The Expression of Insulin-Like Growth Factor II Messenger RNA-Binding Protein 3 Upregulated in Intradural Extramedullary Schwannomas

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Abstract:

Introduction: Tumor size is an important factor in determining the appropriate clinical management of intradural-extramedullary schwannoma. A tumor volume reduction may be achieved by conservative targeted therapy instead of invasive surgery if a molecular event related to tumor size is discovered. Insulin-like growth factor II messenger RNA-binding protein 3 (IMP3), an oncofetal tumor-associated antigen that is expected to be a target for immunotherapy, was focused on in this study.

Methods: The IMP3 status was assessed by immunohistochemistry in 64 samples of intradural-extramedullary schwannoma, and the correlation between IMP3 expression and tumor size was evaluated.

Results: Immunohistochemically, high IMP3 expression was observed in ～85% of schwannomas. The maximum tumor diameter of the high IMP3 expression group was significantly larger than that of the low IMP3 expression group (34.3 mm vs 18.5 mm, p=0.002). The receiver operating characteristic curve demonstrated that a maximum tumor diameter of 24 mm was a predictable factor for IMP3 expression (sensitivity, 0.7; 1−specificity, 0.2; area under the curve, 0.82).

Conclusions: Upregulated IMP3 expression was associated with large tumor size, suggesting a possible therapeutic approach.

Keywords:

schwannoma, IMP3, intradural-extramedullary tumor, targeted therapy

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Introduction

Schwannomas are typically benign tumors that arise from Schwann cells of the nerve sheath[1)]. Spinal schwannomas cause few subjective symptoms because of their slow growth. However, large lesions present aggressive behavior with local pressure on the nerves and spinal cord. Spinal schwannoma with sensory or motor disturbance requires tumor resection in combination with a laminotomy. Patients with sacral lesions or multiple schwannomas require invasive surgery although minimally invasive procedures are preferable because of the potential functional damage[2)]. However, tumor volume reduction may be achieved by conservative targeted therapy if a molecule related to tumor size is identified. However, no well-characterized upregulated protein was noted in schwannoma.

Insulin-like growth factor II messenger RNA-binding protein 3 (IMP3), an oncofetal tumor-associated antigen, is expected to be a target for antigen-specific cancer immunotherapy[3)]. IMP3 binds to the 5' untranslated region of IGF-II leader-3 mRNA as a translational activator, controlling cell proliferation[4)]. IMP3 is a prognostic biomarker for several malignant tumors including renal cell carcinoma and hepatocellular carcinoma but is expressed at low or undetectable levels in normal mature tissues and benign tumors[5-10)]. It is believed that little is known about IMP3 expression patterns in schwannoma.

Previous studies revealed that the RAF/mitogen-activated

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Table 1. Clinicopathological Parameters of 64 Schwannomas.

Variable	Group	No.
Sex	Male	32
	Female	32
Age (12–87, mean: 53)	<53	30
	≥53	34
Tumor maximum diameter (6–140, mean: 26)	<26 mm	29
	≥26 mm	35
Vertebral column	Cervix	18
	Thoracic	23
	Lumbar	17
	Sacrum	6
Schwannomatosis	Yes	11
	No	53
Histological subtype	Conventional	59
	Cellular	5

[Abbreviations] No., number of patients

kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway was activated in schwannomas[11,12], and IMP3 activated MEK/ERK signaling in the progression of several cancers[13,14]. This study aims to investigate the IMP3 expression in schwannoma to confirm its value as a candidate for targeted therapy.

Materials and Methods

Patients and tissue samples

The current study included 64 tumors obtained from 64 cases diagnosed as schwannoma. The samples were surgically resected tumors, and all surgeries were performed at a single facility between 2007 and 2012. The maximum tumor diameter was measured by preoperative magnetic resonance imaging. The diagnosis was confirmed by experienced bone and soft-tissue pathologists following the most recent World Health Organization (WHO) classification. The histological subtypes of the tumors, conventional or cellular variants, were also determined. Conventional schwannomas are characterized by the proliferation of spindle-shaped cells with Verocay bodies (Antoni A) accompanied by loose cellular myxoid stroma (Antoni B). A cellular schwannoma has predominantly cellular growth but no Verocay bodies[1]. The tumor was judged as cellular schwannoma rather than the conventional variant in the current study if the area of high cellularity was conspicuous or if mitotic figures were occasionally detected.

Immunohistochemistry

Immunohistochemical staining was performed as described[15]. Antigen retrieval was undertaken by boiling the slides with Target Retrieval Solution (Dako, Carpinteria, CA, USA). Mouse primary antibodies against anti-CLDN 6 (1:50 dilution) and Ki-67 (MIB-1) (1:100 dilution) (Dako) were used. The immune complex was detected with the EnVision Detection System (Dako) and the labeled antigens were visualized by 3,3'-diaminobenzidine tetrahydrochloride as a chromogen. A lymphoid follicle was used as a positive control for IMP3.

The immunohistochemical expression of IMP3 was evaluated as previously described[16]. Cytoplasmic staining was interpreted following the maximum area of staining. The proportion of cells immunoreactive for IMP3 was classified subjectively as 0 (absent staining), +1 (1%-49%), and +2 (> 50%). The staining intensity for IMP3 was considered strong if it was easily observed at a lower-power magnification and weak if it was fuzzy at lower-power magnification. The intensity was scored as follows: 0=absent staining, +1= weak, and +2=strong. A total score (proportion score+intensity score) of +3 or +4 and less than +2 were considered to be high and low expressions, respectively.

The MIB-1 labeling index was defined as the percentage of immunoreactive cells divided by the total number of cells in the evaluated area. Five viable fields from the area of maximal labeling were chosen for counting.

Statistical analysis

Continuous variables are presented as the means±standard deviation. The chi-square or Fisher's exact tests were used as appropriate to evaluate the association between two variables. A two-sided p value of <0.05 was considered significant. A receiver operating characteristic (ROC) curve was created to predict IMP3 expression about the maximum tumor diameter. The data analysis was conducted with the JMP statistical software package (ver. 9.0.2; SAS Institute, Cary, NC, USA).

Results

Clinicopathological findings

The clinicopathological parameters of 64 schwannomas are summarized in Table 1. The patients included 32 men and 32 women with an average age of 53 years (range, 12-87 years) at the time of surgery. The median maximum diameter of the tumors was 26 mm (range, 6-140 mm). The vertebral column of tumors was cervical vertebrae (18 cases), thoracic vertebrae (23 cases), lumbar vertebrae (17 cases), and sacrum (six cases). The center of the tumor was evaluated as the vertebral level if a tumor covered several vertebral levels. Etiologically, 11 of 64 cases were diagnosed as multiple schwannomas. Histopathologically, 59 and five conventional and 5 cellular schwannomas, respectively, were included in the current study.

Immunohistochemistry

Representative hematoxylin-eosin and immunohistochemical staining for IMP3 are illustrated in Fig. 1. Serial sections stained with hematoxylin-eosin and immunohistochemical staining for IMP3 and S-100 protein were shown in Fig. 2. The expression results are summarized in Table 2 and Fig.

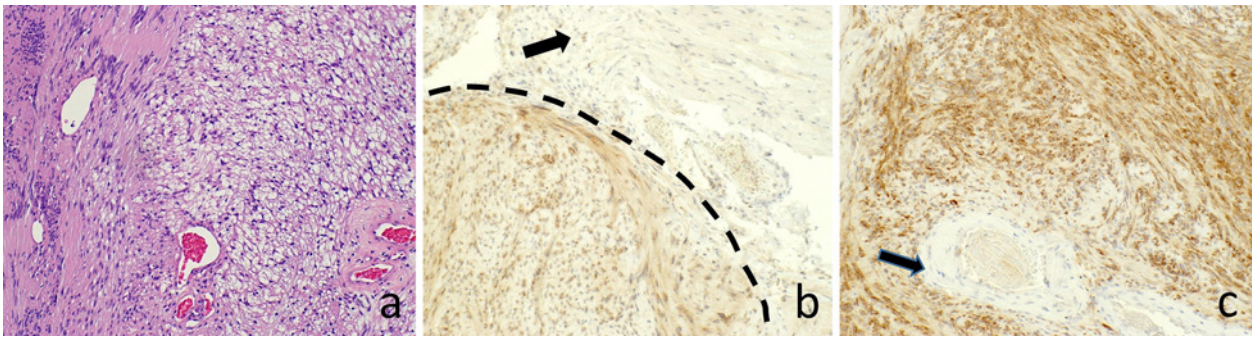

Figure 1. Hematoxylin–eosin (a) and immunohistochemical (b and c) staining of schwannomas. Most schwannoma cells are positive for IMP3. Mature neural (b, *arrow*) and endothelial (c, *arrow*) cells are negative for IMP3 (original magnification ×100).

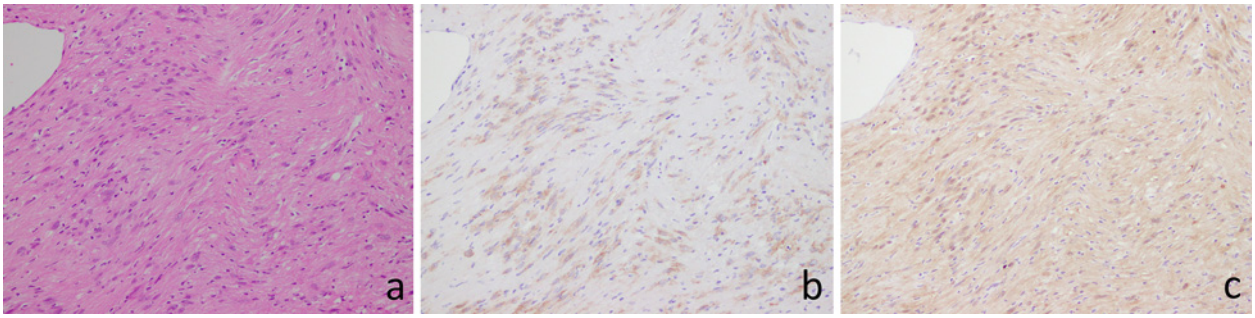

Figure 2. Serial sections stained with hematoxylin-eosin (a), IMP3 (b) and S-100 protein (c) (Original magnification ×200).

Table 2. Results of Immunohistochemical Staining for IMP3.

	Positive				Negative		
Score	4	3	3	Total	2	0	Total
Proportion	2 (diffuse)	2 (diffuse)	1 (focal)		1 (focal)	0	
Intensity	2 (strong)	1 (weak)	2 (strong)		1 (weak)	0	
Schwannoma (*n*=64)	41 (64.1%)	10 (15.6%)	3 (4.7%)	54 (84.4%)	6 (9.3%)	4 (6.3%)	10 (15.6%)

3. High IMP3 expression was observed in 54 (84.4%) of 64 schwannomas (diffuse strong, 41; diffuse weak, 9; focal strong, 4), whereas 10 (15.6%) of 64 schwannomas showed low expression (focal and weak, 6; no expression, 4). No difference in the result of IMP3 staining was noted between the two components because the staining proportion and intensity of Antoni A were equal to those of Antoni B. From the point of the maximum tumor diameter, 33 (94.3%) of 35 cases with large tumors (≥26 mm) and 21 (72.4%) of 29 cases with small tumors (<26 mm) showed high IMP3 expression. The positivity ratio in the large tumor (≥26 mm) group was significantly higher than that of the small tumor (<26 mm) group (p=0.03). A case-control study that compared the large tumor group with the small tumor group was performed (Table 3). Thus, only the IMP3 high/low parameter was relevant to tumor size (p=0.03). A multivariate analysis (IMP3 high/low adjusted by age) was conducted and determined that the IMP3 score was significantly correlated with tumor size (odds ratio, 6.5; 95% confidence interval, 1.2-34.2; p=0.02) (Table 4).

As for the vertebral column, 17 (94.4%) of 18 cases in the cervical vertebrae, 18 (78.3%) of 23 cases in the thoracic vertebrae, 13 (76.5%) of 17 cases in the lumbar vertebrae, and six (100%) of six cases in the sacrum showed high IMP3 expression. Etiologically, 10 (90.9%) of 11 multiple schwannomas and 44 (83%) of 53 solitary schwannomas showed high IMP3 expression. Histopathologically, 49 (83.1%) of 59 conventional schwannomas and five (100%) of five cellular schwannomas showed high IMP3 expression.

The average MIB-1 labeling index was 5% (range, 1%-10%). IMP3 positivity and high MIB-1 index were not correlated with each other.

Correlation between tumor size and high IMP3 expression

The maximum tumor diameter of the high and low IMP3 groups was 34.3±21.1 and 18.5±7.4 mm, respectively. The tumor size in the high IMP3 expression group was significantly larger than that of low-expression group (p=0.002;

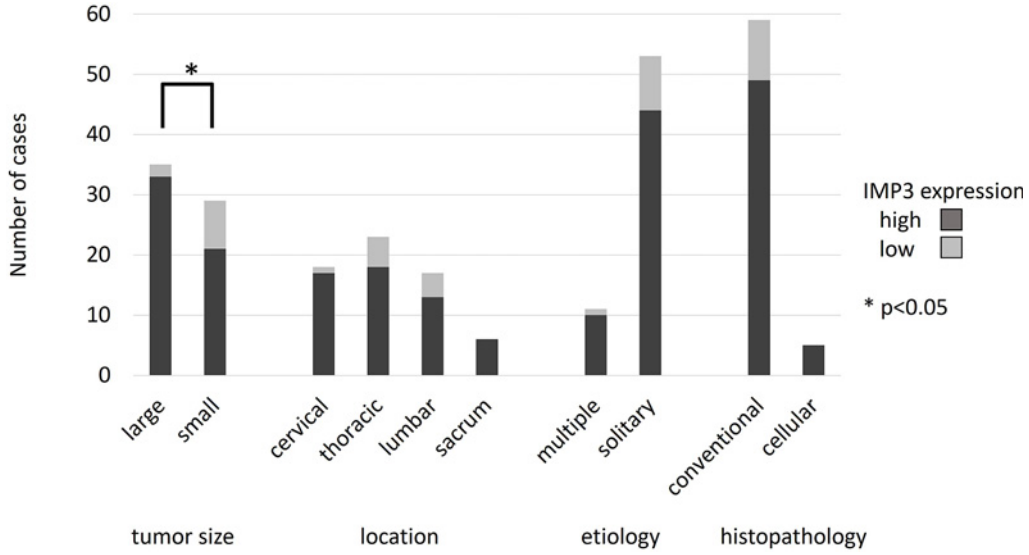

Figure 3. Distribution of IMP3 expression following clinicopathological parameters. IMP3 expression in the large tumor (≥26 mm) group was significantly higher than that of the small tumor (<26 mm) group.

Table 3. Univariate and Multivariate Analysis Comparing Small and Large Tumor Size Groups.

Variables	≤25 mm (*N*=29)	≥26 mm (*N*=35)	*P* value
Age (years)	54.7±16.5	51.5±18.1	0.58
Sex (male, female)	15/14	17/18	1.00
IMP3 high/low	21/8	33/2	0.03
Multiple schwannomatosis +/−	8/21	3/32	0.05
Subtype conventional/cellular	27/2	32/3	1.00

Table 4. Logistic Regression Model.

Variable	Large tumor size/ total cases (*n*)	Odds ratio (95% CI)	*P* value
IMP3 expression [a]			
high	33/54	6.53 (1.2–34.1)	0.02
low	2/10		

IMP3 Insulin-like growth factor II messenger RNA-binding protein 3

a The variable was adjusted by age.

Fig. 4a). ROC curve demonstrated that a maximum tumor diameter of 24 mm was a predictable factor for IMP3 expression, which maximized sensitivity (true positives) and minimized 1−specificity (false positives; Fig. 4b). When the cutoff value was 24 mm, sensitivity was 0.7 and 1−specificity was 0.2. The area under the curve was 0.82, indicating that this cutoff was highly accurate in predicting high IMP3 expression.

Discussion

The present study aims to identify an upregulated biomarker related to tumor size in schwannoma. IMP3 was focused on and its expression was evaluated by immunohistochemistry. IMP3 expression was elevated in ~85% of schwannomas and was correlated with large tumor size. The maximum tumor diameter was a significant factor for high IMP3 expression with a cutoff value of 24 mm.

Spinal schwannomas are treated surgically and do not recur by gross total resection if they cause sensory or motor disturbance if growing intraspinally. However, invasive surgical procedures are needed to remove the sacral lesions. In addition, patients with multiple schwannomas sometimes have a plurality of surgeries, which may lead to functional spine damage. Tumor size seems to be an important factor in determining the appropriate clinical management. The current study postulated that if a molecular event related to the tumor size can be identified, volume reduction may be

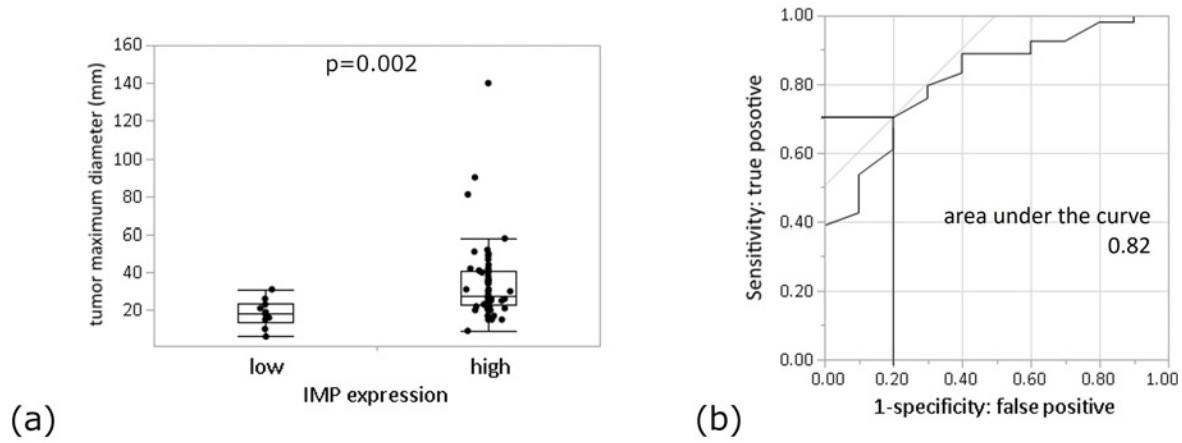

Figure 4. (a) Comparison of maximum tumor diameter between the high and low IMP3-expressing groups. The diameter in the high IMP3 expression group was significantly greater than that in the low-expression group. (b) ROC curve demonstrated that a maximum tumor diameter of 24 mm was the threshold value for high IMP3 expression.

achieved by the targeted therapy. Agnihotri et al. analyzed the global gene sequence and revealed that the RAF/MEK/ERK pathway was activated in some schwannomas[11]. Ammoun et al. also reported MEK/ERK pathway activation and its role in schwannoma growth[12]. In addition, a recent study demonstrated that IMP3 activated MEK/ERK signaling in the progression of colorectal or prostate cancer[13,14], Considering these backgrounds, IMP3 is focused on and the expression pattern of IMP3 in schwannomas is evaluated.

IMP3 is ubiquitously expressed during embryogenesis, and many malignancies show aberrant IMP3 overexpression[5-7]. A recent clinical trial evaluated the effect of peptide vaccination against head and neck squamous cell cancer, and peptides including IMP3 were used in this trial[17]. Consequently, the patient exhibited a complete response after the vaccination. The current study showed that the IMP3 expression was elevated in ～85% of schwannomas. This is the first report to indicate that IMP3 expression was elevated in a benign tumor because previous articles showed that IMP expression in reactive lesions and benign neoplasms were generally low (0%-30%) by immunohistochemical analysis[9,10]. The co-authors of the current study even reported that schwannoma arising in the stomach showed negative staining for IMP3[16]. However, the gap in IHC results was certain between these two types of schwannomas because the origin of gastric schwannoma is completely different from that of the spine[18]. As described above, the MEK/ERK pathway was activated in schwannomas, and IMP3 activated MEK/ERK signaling in the progression of several cancers. To suggest a possible therapeutic approach, additional studies are needed to verify the correlation between IMP3 and the pathway in spinal schwannomas. Mancarellae et al. reported ATP-binding cassette sub-family F member 1 (*ABCF1*) mRNA, a target of IMP3, and concluded that high IMP3 expression together with lower *ABCF1* expression resulted in poor prognosis[19]. This molecule may be a candidate for further study to clarify the molecular event relevant to IMP3.

The current study had several limitations. The IMP3 mechanism in schwannoma is unclear. In the current study, the MIB-1 labeling index was not influenced by IMP3, which suggests the absence of correlation between IMP3 and proliferation in schwannomas. However, Ki-67 antigen is variably expressed depending on the phase of the cell cycle, and the estimation of growth fraction only by the MIB-1 labeling index may not be sufficient to measure proliferative activity[20,21]. Additional studies are needed to verify the growing mechanism of schwannomas. The current study yielded evidence that large tumor size was a significant risk factor for high IMP3 expression and 24 mm was the threshold value. This result suggests the possibility that IMP3 is involved in tumor progression. However, tumor size does not necessarily mean the capacity of tumor growth. The tumor size depends on the timing of excision during the tumor growth. Furthermore, small tumors cannot be detected because they do not cause neurological symptoms until they become large enough. This means that the tumor size is influenced by various factors. Therefore, a limitation was noted in effectively identifying causative factors or genes that are related to the tumor size or growth. Currently, the current study cannot conclude which hypothesis is appropriate regarding whether IMP3 is upregulated because of tumor growth or because higher IMP3 expression causes tumor growth. However, epigenetic alternations in spinal schwannoma have not been reported. In addition, the proportion and intensity of IMP3-positive cells in 90% of cases were monotonous (diffuse or weak staining type) in the current study, which implied fewer epigenetic alternations in spinal schwannomas. Considering these backgrounds, higher IMP3 expression may cause tumor growth.

In conclusion, upregulated IMP3 expression was observed in ～85% of schwannomas. High IMP3 expression was correlated with large tumor size, which suggests that IMP3 may be a candidate for targeted therapy and lead to the volume reduction of schwannomas. These cases may benefit from targeted therapy because invasive surgical procedures are required to cure sacral lesions or patients with multiple

schwannomas.

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Informed Consent: Opt-out consent

References

1. Antonescu CR, Perry A, Woodruff JM. Schwannoma. Lyon (France): IARC; 2013. WHO Classification of Tumours of Soft Tissue and Bone. p. 170-4.
2. Harimaya K, Matsumoto Y, Kawaguchi K, et al. Clinical features of multiple spinal schwannomas without vestibular schwannomas. J Orthop Sci. 2021;23;S0949-2658(21):00089-0.
3. Hirayama M, Tomita Y, Yuno A, et al. An oncofetal antigen, IMP-3-derived long peptides induce immune responses of both helper T cells and CTLs. Oncoimmunology. 2016;5(6):e1123368.
4. Liao B, Hu Y, Brewer G. RNA-binding protein insulin-like growth factor mRNA-binding protein 3 (IMP-3) promotes cell survival via insulin-like growth factor II signaling after ionizing radiation. J Biol Chem. 2011;286(36):31145-52.
5. Jiang Z, Chu PG, Woda BA, et al. Analysis of RNA-binding protein IMP3 to predict metastasis and prognosis of renal-cell carcinoma: a retrospective study. Lancet Oncol. 2006;7(7):556-64.
6. Jeng YM, Chang CC, Hu FC, et al. RNA-binding protein insulin-like growth factor II mRNA-binding protein 3 expression promotes tumor invasion and predicts early recurrence and poor prognosis in hepatocellular carcinoma. Hepatology. 2008;48(4):1118-27.
7. Mueller-Pillasch F, Pohl B, Wilda M, et al. Expression of the highly conserved RNA binding protein KOC in embryogenesis. Mech Dev. 1999;88(1):95-9.
8. Hao S, Smith TW, Chu PG, et al. The oncofetal protein IMP3: a novel molecular marker to predict aggressive meningioma. Arch Pathol Lab Med. 2011;135(8):1032-6.
9. Shi M, Fraire AE, Chu P, et al. Oncofetal protein IMP3, a new diagnostic biomarker to distinguish malignant mesothelioma from reactive mesothelial proliferation. Am J Surg Pathol. 2011;35(6): 878-82.
10. Hanley KZ, Facik MS, Bourne PA, et al. Utility of anti-L523S antibody in the diagnosis of benign and malignant serous effusions. Cancer. 2008;114(1):49-56.
11. Agnihotri S, Jalali S, Wilson MR, et al. The genomic landscape of schwannoma. Nat Genet. 2016;48(11):1339-48.
12. Ammoun S, Ristic N, Matthies C, et al. Targeting ERK1/2 activation and proliferation in human primary schwannoma cells with MEK1/2 inhibitor AZD6244. Neurobiol Dis. 2010;37(1):141-6.
13. Zhang M, Zhao S, Tan C, et al. RNA-binding protein IMP3 is a novel regulator of MEK1/ERK signaling pathway in the progression of colorectal cancer through the stabilization of MEKK1 mRNA. J Exp Clin Cancer Res. 2021;40(1):200.
14. Sjekloča N, Tomić S, Mrklić I, et al. Prognostic value of IMP3 immunohistochemical expression in triple negative breast cancer. Med (Baltim). 2020;99(7):e19091.
15. Bekki H, Yamamoto H, Takizawa K, et al. Claudin 6 expression is useful to distinguish myxofibrosarcomas from other myxoid soft tissue tumors. Pathol Res Pract. 2017;213(6):674-9.
16. Yamamoto H, Arakaki K, Morimatsu K, et al. Insulin-like growth factor II messenger RNA-binding protein 3 expression in gastrointestinal mesenchymal tumors. Hum Pathol. 2014;45(3):481-7.
17. Yoshitake Y, Fukuma D, Yuno A, et al. Phase II clinical trial of multiple peptide vaccination for advanced head and neck cancer patients revealed induction of immune responses and improved OS. Clin Cancer Res. 2015;21(2):312-21.
18. Walsh NM, Bodurtha A. Auerbach's myenteric plexus. A possible site of origin for gastrointestinal stromal tumors in von Recklinghausen's neurofibromatosis. Arch Pathol Lab Med. 1990;114(5): 522-5.
19. Mancarella C, Pasello M, Ventura S, et al. Insulin-like growth factor 2 mRNA-binding protein 3 is a novel post-transcriptional regulator of Ewing sarcoma malignancy. Clin Cancer Res. 2018;24 (15):3704-16.
20. Brown DC, Gatter KC. Monoclonal antibody Ki-67: its use in histopathology. Histopathology. 1990;17(6):489-503.
21. Sasaki K, Murakami T, Kawasaki M, et al. The cell cycle associated change of the Ki-67 reactive nuclear antigen expression. J Cell Physiol. 1987;133(3):579-84.